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PURIFICATION PROCESS FOR MANUFACTURING A ~~HIGH~~ HIGHLY PURE ACARBOSE

Background of the Invention

1. ~~Filed~~ Field of the Invention

The present invention relates to a process for manufacturing ~~high~~ a highly pure acarbose, ~~more particularity,~~ and particularly to a process which uses alcohol for precipitation and separation, and a ~~strongly~~ strong cation exchange chromatography and an immobilized enzyme affinity chromatography for manufacturing a ~~high pure~~highly pure acarbose to treat diabetes.

2. Description of the Related Art

Acarbose,

O-4,6-Dideoxy-4-[[[1S-(1α,4α,5α,6α)]-4,5,6,-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]-α-D-glucopyranosyl-(1 → 4)-O-α-D-glucopyranosyl-(1 → 4)-D-glucose,$C_{25}H_{43}NO_{18}$,Mw 645.63,~~it~~ is an oligo-~~derivatives~~derivative. ~~The acarbose~~ Acarbose inhibits the activity of α-glucosidase at the edge ~~in~~ of the small intestine by invertibility for slowly turning complex carbohydrates ~~complex~~ and disaccharide into glucose, which can be absorbed by ~~human~~

humans, to decrease concentration of triglycerol and insulin in blood and blood sugar.

In the early ~~1970~~1970s, ~~the~~ acarbose could improve the ratio of meat and fat, so it ~~uses~~ was used ~~to add~~ as an additive in ~~the feed to~~ feed ~~the~~ for animals such as ~~pig~~pigs. Recently, ~~the researches~~ researchers have found that ~~find the~~ acarbose ~~could control~~ controls the blood sugar of NIDDM and decreases the insulin value after ~~diet~~ eating, for preventing diabetic cardiovascular ~~complication~~complications~~, but it~~. However, acarbose cannot ~~can not~~ directly change ~~the~~ insulin resistance. ~~The acarbose~~ Acarbose only has a few aftereffects, such as abdominal distension, borborygmus and diarrhea, ~~being~~ which go away after ~~treating~~ a period of treatment, and ~~it~~ hardly ~~affects~~ affect ~~the~~ health. The glucobay of the Bayer ~~is~~ was first approved ~~firstling~~ in 1995 by FDA. So far, ~~the~~ acarbose is primarily manufactured using ~~manufacturing acarbose is mainly used~~ *Actinoplanes sp.* or *Streptomyces glaucescens*.

The DOH of Taiwan adjusts the adjusting blood sugar material to be health food. Further, ~~the fat reason of the Easterner~~ Easterners ~~is eating~~ eats polysaccharide which differs ~~different~~ from the ~~westerner is eating~~ fat eaten by Westerners. Therefore, ~~the~~ acarbose not only treats diabetes, but can also ~~uses~~ be used in ~~the~~ diet food.

US. Pat. 4,062,950 ~~of~~ sets forth a recover and purification process for manufacturing acarbose, and discloses that acarbose-containing fermentation broth is discolored by anion resins or activated carbons in the ~~acid~~ acidic condition, and that acarbose are absorbed by activated carbons in the neutral condition and are eluted by ethyl alcohol solution or acetone solution in the ~~acid~~ acidic condition. The elute passes through the cation exchange chromatography, and acarbose are finally washed by the acid or base solution. The eluted liquid is counteracted and concentrated in the vacuum, and ~~the 85% purity of the~~ acarbose with 85% purity is precipitated by the organic solvent. The high purity of the acarbose can be manufactured if the exchange chromatography uses celluloses ~~to be~~ as a matrix. Further, the liquid is concentrated and precipitated by the organic solvent to get a ~~high purity of the~~ highly pure acarbose. The process is ~~complicate~~ complicated because the process must use ~~the~~ activated carbons for absorbing and the exchange chromatography process repeated many times for purification of acarbose.

US. Pat. 4,174,439 mixes cation and anion exchange resin into acarbose-containing fermentation broth to absorb acarbose and elutes the acarbose by deionized water. The acarbose solution ~~process~~ is processed twice by a cation and anion exchange resin and is eluted by hydrochloric acid, and

~~process~~ is processed with a neutralizing treatment by an anion exchange resin and frozen until dry to ~~get 52%-58% purity of the~~ acarbose with 52-58% purity.

Further, US. Pat. 4,666,776 and US. Pat. 4,767,850 improve US. Pat. 4,174,439 to use ~~strongly cation~~strong cation exchange resin ~~and~~, be washed by hydrochloric acid, ~~and~~ be processed by a ~~process~~ neutralizing treatment ~~by~~ with an anion exchange resin and frozen until dry to get ~~79%-82% purity of the~~ acarbose with 79-82% purity.

The above ~~mentions~~ methods of purifying the acarbose ~~process~~ all repeat the anion and cation exchange chromatography to get the acarbose solution and finally use cation exchange chromatography to get a high concentration of acarbose. ~~But~~However, it is difficult to achieve a ~~the~~ purity of acarbose ~~is hard to be~~ sufficient for use as a medical drug.

US. Pat. 4,904,769 discloses a method in which ~~taking~~ impure acarbose passes through a ~~weakly~~ weak cation exchange chromatography containing carbonyl, cellulose, and dextran ~~in~~ with specific temperature and pH ~~value~~ values to get ~~90% purity~~ acarbose with a 90% purity. The process is complicated and uses ~~weakly~~ weak ion exchange chromatography ~~being~~ in an expansive process, resulting in high manufacturing ~~cost~~costs.

~~And~~Finally, WO~~.~~ 99/07720 discloses ~~which~~ taking an impure acarbose ~~manufacturing~~ manufactured by US. Pat. 4,174,439, US. Pat. 4,666,776 and US. Pat. 4,767,850, which passes through a ~~strongly cation~~strong cation exchange chromatography containing non-aromatic to get ~~high pure~~highly pure acarbose, and the processes are typically ~~has~~ complicated ~~process and~~ with high manufacturing ~~cost~~costs.

Summary of the Invention

~~According to~~ As discussed above ~~mention~~, the present invention improves the complicated process and high manufacturing ~~cost~~costs of the prior art, and ~~get~~ achieves a ~~high pure~~highly pure acarbose ~~being~~ appropriate for use as a medical drug.

The present invention considers processes and material of the above ~~mentions~~ mentioned prior art, to improve an impure acarbose manufacturing process which applies the solubility between the acarbose and alcohol or methyl alcohol ~~and~~, absorbs the acarbose by ~~strongly~~ strong exchange resin, and eliminates like acarbose by sodium chloride and ammonia solution. The ~~75%-80% purity~~ acarbose with a 75-80% purity ~~could get~~ can be achieved by eluting high concentration ammonia solution, and finally ~~passes~~ passing through

an α-glucosidase column to get up 95% ~~purity~~ pure acarbose, ~~to overcome~~ thereby overcoming the high manufacturing cost ~~and~~ costs complicated processes of the prior art.

Further scope of the applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

Brief Description of the Drawings

~~The accompanying drawing is included to provide a further understanding of the invention, and is incorporated in and constitutes a part of this specification. The drawing illustrates an embodiment of the invention and, together with the description, serves to explain the principles of the invention. In the drawing,~~The present invention will become more fully understood from the detailed description given hereinbelow and the accompanying drawings

which are given by way of illustration only, and thus are not limitative of the present invention, and wherein:

Fig. 1 is a flow chart showing a purification process for manufacturing a ~~high-pure~~highly pure acarbose of the present invention;

Fig. 2 is flow chart showing a purification process for manufacturing a ~~high-pure~~highly pure acarbose of Example 1 of the present invention;

Fig. 3 is flow chart showing a purification process for manufacturing a ~~high-pure~~highly pure acarbose of Example 2 of the present invention;

Fig. 4 is flow chart showing a purification process for manufacturing a ~~high-pure~~highly pure acarbose of Example 3 of the present invention; and

Fig. 5 is flow chart showing a purification process for manufacturing a ~~high-pure~~highly pure acarbose of Example 4 of the present invention.

Detailed Description of the preferred Embodiments

Refer to Fig. 1, the present invention discloses a purification process for manufacturing ~~high-pure~~highly pure acarbose ~~comprises~~ comprising the steps of:

~~step~~ Step 10, start;

~~step~~ Step 15, adding alcohol in an acarbose-containing fermentation broth for precipitation;

~~step~~ Step 20, passing sediments through ~~strongly cations~~strong cation exchange resin and processing an immobilized enzyme affinity chromatography process.

The present invention discloses a ~~purification and~~ process for purifying acarbose ~~process~~ from ~~acarbose containing~~ acarbose-containing fermentation broth to get a ~~high pure~~highly pure acarbose to treat diabetes. The ~~strongly cations~~strong cation exchange chromatography uses styrene divinylbenzene copolymer without methoxymethylmethacrylamide to be a resin matrix, and the enzyme of the immobilized enzyme affinity chromatography uses α-amyloglucosidase(α-glucoamylase).

Further, an upper liquid of the acarbose-containing fermentation broth is made by centrifugal effect or filter and concentrates 1/10 volume by a rotary ~~evaporators~~ evaporator concentrating system. Then, adding adequate ethyl alcohol solution or methyl alcohol solution takes an upper liquid by centrifugating, and the upper liquid ~~concentrates to consistency~~forms a concentrate. Finally, the ~~consistency~~ concentrate uses ethyl alcohol to get a sediment ~~of the~~ containing acarbose, and the sediment is ~~solved~~ dissolved by distilled water to be in a 200 mg/mL concentration ~~and~~. The pH of the

dissolved sediment is adjusted to a level of approximately 5-9, ~~adjusts pH=5-9~~ to be a mixing liquid.

The process of ion exchange resin ~~describes using strongly cation~~uses a strong cation exchange resin, such as AMBERJET 1200 H resin or AMBERJET 1200 Na (Rohm and Hass Company), and is washed by deionized water ~~till~~ until the pH value of ~~an~~ the upper liquid is ~~large~~ larger than 4. Then, ~~taking~~ the strong cation exchange resin containing 20-200 mg sugar/mL ~~adds~~ is added into the mixing liquid and ~~blending~~ blended for 10~30 minutes~~, and taking a~~. A part of the resin is then washed several times ~~by~~ with distilled water. The resin is then washed by NaCl to ~~get~~ obtain a lot of acarbose-like sugars, and is eluted by an 0.75N ammonia solution. Finally, the resin is ~~solved~~ dissolved by a 1.5N ammonia solution to get acarbose, ~~and concentrating the acarbose and~~ which is concentrated and precipitated using ethyl alcohol to get a precipitation in which the purity of acarbose is 75~80%.

~~The~~ Adequate distilled water is added to the impure acarbose powders ~~add adequate distilled water~~ to adjust the pH value to between five and nine, ~~and passes~~ which is then passed through a column containing AMBERJET 4400 OH resin and α-amyloglucosidase. Firstly, the column is washed with distilled water having one to four times the volume as column and ~~solves~~ a temperature of

55~75°C ~~distilled water~~. Then, ~~collecting~~ the acarbose concentrates are collected, and ~~uses the~~ ethyl alcohol is used to obtain ~~to get~~ a sediment. The sediment is then cooled and dried to ~~get the~~ increase the purity of the acarbose up to 95% ~~acarbose~~.

Example 1:

~~Refer~~ Referring to Fig. 2, the present invention comprises the following steps ~~of~~:

Step 100~~, eliminating mycelium~~: mycelium is eliminated from acarbose-containing fermentation broth by ~~centrifugating~~ centrifugation or ~~filtering~~filtration;

Step 102~~,~~: filtrate ~~concentrating filtrate~~ or an upper liquid (1000 ml) of the centrifuged acarbose-containing fermentation broth ~~to be consistency~~ are concentrated by a ~~concenteration~~ concentrating system;

Step 104~~, adding~~: adequate ethyl alcohol is added to the ~~consistency~~ concentrate and ~~blending to be~~ blended into a solution;

Step 106~~, taking~~: an upper liquid is taken from the solution by centrifugation after blending for 30 minutes ~~by centrifugating~~;

Step 108, ~~concentrating~~: the upper liquid of the centrifuged solution is further concentrated ~~to be a consistency~~ by the concentrating system;

Step 110, ~~taking~~: the ~~consistency~~ concentrate is taken into a 99.9% ethyl alcohol solution, ~~which~~ wherein the amount of ethyl alcohol is ~~nice~~ equal to nine times the volume ~~as the consistency~~ of the concentrate, to ~~get~~ obtain a consistency liquid;

Step 112, ~~taking~~: sediment is removed from the consistency liquid by ~~centrifugating~~ centrifugation and ~~solving~~ the sediment is dissolved by water to ~~get~~ obtain an impure acarbose solution;

Step 114, ~~getting~~: using High Performance Liquid Chromatography (HPLC) to obtain an ~~the~~ impure acarbose solution ~~being~~ with 10%, 1560 mg purity ~~and being 1560 mg by HPLC~~;

Step 116, ~~-~~: blending a ~~strongly cation~~ strong cation exchange resin, such as AMBERJET 1200 H resin (Rohm and Hass Company), with the acarbose solution for 10 minutes, to ~~get~~ obtain a resin;

Step 118, ~~-~~: using a 1.0N sodium chloride solution to eliminate an impurity in the resin;

Step 120, ~~-~~: using a 0.75N ammonia solution to eliminate ~~a rest of the~~ further impurities ~~impurity~~ in the resin; and

Step 122~~,~~: eluting the resin with a 1.5N ammonia solution to obtain ~~get a high pure~~highly pure acarbose, in which the purity of the acarbose is 60%, 1220 mg.

Example 2:

~~Refer~~ Referring to Fig. 3, the present invention comprises the following steps ~~of~~:

Step 200~~,~~: adjusting the pH value of an impure acarbose to between six and seven ~~of an impure acarbose~~;

Step 202~~,~~: adding ~~an~~ a cation exchange resin containing 250 mg sugars/g ~~resin~~ into the impure acarbose, in which the resin is AMBERJET 1200 Na resin (Rohm and Hass Company), ~~into the impure acarbose~~ in order to ~~get~~ obtain a solution;

Step 204~~,~~: blending the solution for 10 minutes and taking ~~an~~ the upper liquid;

Step 206~~,~~: adding a strong cation exchange resin containing 80 mg sugars/mL into the upper liquid, in which the resin is AMBERJET 1200 H resin (Rohm and Hass Company) ~~into the upper liquid~~, to ~~get~~ obtain a mixing solution;

Step 208~~,~~: mixing and shaking the mixing solution for 10 minutes to make the strong cation exchange resin ~~absorbing~~ absorb acarbose;

Step 210~~,~~: using a 1.0N sodium chloride solution to eliminate an impurity in the acarbose; and

Step 212~~,~~: using an ammonia solution to eliminate ~~the rest of an impurity~~ further impurities in the acarbose to ~~get~~ obtain a ~~high pure~~highly pure acarbose~~, which the~~ having a purity of ~~acarbose is~~ 78%, 1100 mg.

Example 3:

~~Refer~~ Referring to Fig. 4, the present invention comprises the following steps ~~of~~:

Step 300~~,~~: adjusting the pH value ~~between six and seven~~ of an upper liquid from an impure acarbose ~~mixing~~ mixed with a strong cation exchange resin to between six and seven;

Step 302~~,~~: passing the upper liquid through a strong cation exchange resin column, 8×50 cm, containing AMBERJET 1200 H resin (Rohm and Hass Company) and washing the strong cation exchange resin in the column ~~by~~ with deionized water ~~till~~ until the absorbance of the strong cation exchange resin ~~being~~ is zero or steady;

Step 304~~,~~: getting ~~an~~ acarbose-containing ~~fractions~~ fragments by using ~~gradient~~ a gradiated 0.5~1.5N ammonia solution to ~~solve~~ dissolve the strong cation exchange resin;

Step 306~~,~~: concentrating the acarbose to ~~be~~ a certain volume by a ~~concentoration~~ concentrating system; and

Step 308~~,~~: using alcohol ~~for precipitation~~ to precipitate the acarbose to ~~get~~ obtain a ~~high pure~~highly pure acarbose, in which the purity of the acarbose is up 85%, 920 mg.

Example 4:

~~Refer~~ Referring to Fig. 5, the purity of the acarbose powder of the present invention is 85% ~~from~~ as discussed in Example 3 and ~~uses in~~ using this example, ~~comprising~~ the present invention comprises the steps of:

Step 402~~, solving~~: dissolving a powder of acarbose having a ~~, which the~~ purity ~~is~~ of 83%~87%, ~~by~~ using distilled water, to ~~be~~ create a solution;

Step 404~~,~~: adjusting the pH value of the solution to between six and seven ~~of the solution~~;

Step 406~~,~~: passing, with a flow velocity of 1.5mL/min, the solution through an α-amyloglucosidase column, 8×30 cm, containing AMBERJET 4400

OH (Rohm and Hass company) and α-amyloglucosidase, and washing the α-amyloglucosidase column by using a volume of ~~twice times~~ deionized water ~~volume~~ twice that of ~~as~~ the α-amyloglucosidase column or the absorbance being 210 nm and steady;

Step 408, : eluting an acarbose from the α-amyloglucosidase column ~~by~~ using 65°C distilled water;

Step 410, : concentrating the acarbose-containing ~~fractions~~ fragments to be a volume by a ~~concenteration~~ concentrating system; and

Step 412, : using alcohol ~~for precipitation~~ to precipitate the impure acarbose to get a ~~high pure~~highly pure acarbose, ~~which the~~ having a purity of ~~the acarbose is~~ 95%, 900mg.

Advantages of the Invention

The above four examples ~~could get high pure~~can achieve highly pure acarbose ~~to be~~ appropriate for use as a medical drug, ~~and~~ simplify the processes and decrease product costs by using ~~use~~ low-cost resin ~~to decrease the product costs~~.

~~Therefore, the foregoing is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes~~

~~will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described, and accordingly, all suitable modifications and equivalents may be resorted to, falling within the scope of the invention.~~ The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.